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Complement-Fixation Tests on Rabbits with Brown-Pearce Carcinoma.*

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The recent paper by Cheever[1] on complement fixing antibodies in rabbits bearing Brown-Pearce tumors, observed by Kidd,[2] prompts the publication of a similar study carried out in the Spring of 1939 in which similarly positive results were obtained at first, but appeared questionable after the introduction of more rigid controls.

The tumor strain was provided by Dr. J. B. Murphy and Mr. Ernest Sturm. Rabbits were inoculated by intratesticular injection. Antigens were prepared and the complement fixation tests carried out as described by Kidd.[2a]

Twenty-five rabbits in all were tested after inoculation with tumor. In the early tests on some 14 rabbits, sera of 3 out of 6 rabbits in which tumors were rapidly developing with many metastases showed definite complement fixation, as compared with 10 negative normal sera used for control. Two other sera were doubtful and one negative. Of 5 animals with small tumors all were negative; and of 3 animals in which tumors indubitably grew and then regressed, all were negative. It may be of interest to note that in 2 of these latter animals kidney metastases appeared to have attained a size of from 3 to 6 mm or larger and subsequently regressed.

However, difficulties were encountered in attempts to repeat this experiment. A considerable capacity of some normal sera to fix complement was observed under these conditions, and it was noted that the tumor extracts used as antigen also varied markedly in their complement fixing properties. Prolonged centrifugation, filtration through a Mandler filter, or simply passage of time reduced this tendency to fix complement. Attempts to distinguish more clearly between the sera of the normal and tumor-bearing rabbits by altering the strength of the antigen by dilution were unsuccessful, since a dilution of antigen which eliminated all reactions with

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1 Cheever, F. S., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 517.

2 Kidd, J. G., a. PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 292; b. *J. Exp. Med.*, 1940, **71**, 335, 351; c. *J. Bact.*, 1940, **39**, 349.

normal sera usually also obliterated reactions with the sera of tumor-bearing rabbits.

Thus it was evident that the differences to be observed were small, and a more precise type of experiment was adopted. Samples of serum were taken from all rabbits before inoculation with tumor. Frequent, usually weekly bleedings were made from time of inoculation until death from the tumor, and all the samples from any one animal were tested at the same time, thus eliminating most differences due to individual variations in sera and antigens.

Tests were carried out in this way on 12 rabbits. Of 8 rabbits with rapidly progressing, generalized tumors, tests on 3 were doubtful and on 5 negative, and of 4 rabbits with small tumors, all were negative. These animals yielded no certain evidence of the production of complement-fixing antibodies. In no case did we succeed in showing that a serum, negative before inoculation, became definitely positive after inoculation when compared against the same antigen in the same test.

Comment. It is perhaps of significance that in these experiments and those of Cheever, the positive or doubtful results were almost all in animals with rapidly progressing tumors, since it is especially in those animals in which the tumors were regressing that one might expect to find antibody formation, if the formation of antibodies conferred any degree of immunity to the tumor. As sera are diluted only 1-4 in the tests, and positive results were obtained in only 5 of 35 rabbits in Cheever's series, and 3 of 25 rabbits in ours, it is evident that the effects were so slight and infrequent as to be open to several interpretations. Kidd[2b] has reported a few sera reacting in higher dilutions.

Both Kidd and Cheever emphasize the analogy to the serologically active substance extracted from virus-induced rabbit papillomas. Against this would seem to be the strikingly weaker reactions in the rabbits bearing Brown-Pearce carcinomas as compared with those infected with papilloma virus, and also Cheever's observation that various antigenic extracts differ in their specificity when tested with the same positive sera. Unless it is assumed that the reactions are haphazard, it seems probable that a number of antigens are involved. This suggests the possibility that the reactions observed might be due to the production of antibodies to altered antigens of rabbit organs, resulting from pathological processes induced by the tumor, rather than to a single tumor or virus antigen. Kidd noted[2b] that this possibility could not be excluded, and Dmochowski[3] has

[3] Dmochowski, L., *Compt. rendue de la Soc. de Biol.*, 1938, **129**, 349.

shown that sera of rabbits immunized against unheated aqueous extracts of Brown-Pearce carcinoma give cross reactions with pus of rabbits, though not with aqueous extracts of normal organs.

However, if future experiments should conclusively demonstrate the presence of specific tumor antibodies, it would still be necessary to exclude the obvious likelihood (Dmochowski[3]) that the antigenic substance is part of the cellular elements of the tumor, before the virus analogy could logically be pressed. The specificity of tumors such as the one under consideration is comparable to the specificity of transplants in which genetic factors strongly influence the course of growth. These genetic differences must be reflected in chemical differences. Thus it would seem possible that genetic differences between the Brown-Pearce tumor and its host could exist and result in the formation of antibodies. Due to more rapid growth, such antibodies might be more readily formed by a tumor than a transplant.

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Rumen Synthesis of the Vitamin B Complex on Natural Rations.*†

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During the past several years considerable work demonstrating the synthesis of the vitamin B complex in the rumen of polygastric animals has been reported.[1-4] The above mentioned work showed conclusively the synthesis of the vitamin B complex in the rumen when the animal was fed a ration devoid or nearly so of the B complex. What the picture would be if the animal was fed a natural ration containing the members of the B complex has as yet received

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1 Bechdel, S. I., *et al.*, *J. Biol. Chem.*, 1928, **80**, 231.

2 McElroy, L. W., and Goss, H., *Proc. Am. Soc. Biol. Chem.*, 1940, **34**, lxv; *J. Biol. Chem.*, 1939, **130**, 437.

3 Wegner, M. I., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 769.

4 Johnson, P., *et al.*, *J. Dairy Sci.*, 1941, **24**, 57.